Multigeneric resistance to monepantel on a UK sheep farm

Citation for published version:
Bartley, DJ, Hamer, K, Andrews, L, Sargison, N & Morrison A, AA 2019, 'Multigeneric resistance to monepantel on a UK sheep farm', *Veterinary Parasitology*. https://doi.org/10.1016/j.vpoa.2019.100003

Digital Object Identifier (DOI):
10.1016/j.vpoa.2019.100003

Published In:
Veterinary Parasitology

Veterinary Parasitology: X xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Veterinary Parasitology: X

journal homepage: www.journals.elsevier.com/veterinary-parasitology-x

Multigeneric resistance to monepantel on a UK sheep farm

David J. Bartley[a,*], Kim Hamer[b,c], Leigh Andrews[a], Neil D. Sargison[b], Alison A. Morrison[a]

[a] Disease Control, Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, EH26 0PZ, United Kingdom
[b] University of Edinburgh, Royal (Dick) School Veterinary Studies, Easter Bush Veterinary Centre, EH25 9RG, United Kingdom
[c] University of Glasgow, School of Veterinary Medicine, Garscube Estate, Glasgow, G61 1QH, United Kingdom

ARTICLE INFO

Keywords:
Controlled efficacy test
Faecal egg count reduction test
Monepantel
Multigeneric anthelmintic resistance
Sheep

ABSTRACT

The amino acetonitrile derivative, monepantel, represented the first new broad spectrum anthelmintic to be brought to market for use in sheep for over 25 years when it was introduced in 2009. This study characterised monepantel efficacy, using faecal egg count reduction and controlled efficacy tests, against gastrointestinal nematodes following a report of treatment failure in a UK lowland sheep flock. Twelve lambs were each artificially administered 15,000 infective larvae that had been propagated from lamb faeces collected from the farm of interest. The controlled efficacy test showed that a recommended dose rate of monepantel (2.5 mg/kg body weight) administered at day 28 post infection was ineffective at removing the infection in the treated lambs. The result demonstrated simultaneous resistance to monepantel in *Teladorsagia circumcincta*, *Trichostrongylus vitrinus* and *Oesophagostomum venulosum* with efficacies based on adult worm burden reductions, compared to untreated controls, of 78%, 27% and 22% respectively. Monepantel efficacy based on undifferentiated egg count in treated animals, seven day post administration, compared to untreated controls was 85%. The results raise questions about the origins of, and predisposing factors for, resistance development in the three different species, and reinforces the value of differentiating post treatment faecal egg counts to genus or species level.

1. Introduction

Monepantel (Zolvix®) was first registered for use in sheep in 2009 and 2010 in New Zealand and Australia, respectively (Hosking et al., 2010) and subsequently registered for use in other significant sheep producing regions, including the UK. The product was launched in response to the growing threat, and increasing prevalence, of resistance to the three broad-spectrum anthelmintic groups available at the time (Jabbar et al., 2006). Marketing of the product in the UK was accompanied by practical advice on its responsible use to attempt to delay the onset of anthelmintic resistance. The product was recommended, in the UK, for administration during quarantine of introduced animals (Sager et al., 2010) and/or as a mid/late season drench (Leathwick and Hosking, 2009). Unfortunately, resistance to monepantel was reported within a few years of release in New Zealand, Australia, Uruguay, Brazil and the Netherlands (Cintra et al., 2016; Mederos et al., 2014; Playford et al., 2014; Scott et al., 2013; Van den Brom et al., 2015). Monepantel resistance was first reported in the UK in 2018 (Hamer et al., 2018) with *Trichostrongylus vitrinus* being the predominant resistant parasite species, as determined by ITS-2 deep amplicon sequence analysis of post treatment coprocultured L_3. The methods showed the presence/absence of species pre and post monepantel administration (Hamer et al., 2018), but did not provide any information on anthelmintic efficacy at a species level. This study provides the individual species sensitivities to monepantel through characterisation of the field population from the same index case.

2. Materials and methods

2.1. Parasite isolates

Faecal material was collected from lambs from the farm of interest that had been identified through ongoing animal health management (Hamer et al., 2018) and cultured to generate infective larvae (L_3). Larvae were stored in tap water at 4 °C for seven weeks prior to use in the study.

2.2. Experimental design

Twelve worm free lambs were challenged *per os* with 15,000 infective larvae (Day 0). Twenty eight days post infection (PI) monepantel (Zolvix®, Elanco AH) was administered *per os* at the manufacturer's recommended dose rate of 2.5 mg/kg bodyweight (BW) to six lambs. All anthelmintic treatment doses were rounded up to the nearest 1 ml

* Corresponding author.
E-mail address: dave.bartley@moredun.ac.uk (D.J. Bartley).

https://doi.org/10.1016/j.vpoa.2019.100003
Received 25 October 2018; Received in revised form 5 February 2019; Accepted 9 February 2019

(dosage range 2.58–3.09 mg/kg BW). Six lambs were left untreated to act as a control group and to confirm the viability of the infective larvae. Faecal egg counts were monitored on individual faecal samples throughout the trial from day 12 post infection. A modification of the salt flotation faecal egg count method as detailed by Christie and Jackson (1982) was used, the technique has a sensitivity of up to one egg per gram. All of the lambs were slaughtered on day 35 PI and their gastrointestinal tracts removed and processed for worm burden estimation. Total nematode burdens were estimated from counts of 2% subsamples of the abomasal and intestinal washings and saline digests. Enumerated nematodes were classified to stage and species using criteria described in the Ministry of Agriculture, Fisheries and Food document (MAFF, 1986).

2.3. Statistical analysis

Nematode burdens and FECs were square-root transformed to successfully normalise for variance. Burdens were compared using one way ANOVA (Minitab version 13), followed by Fisher's pairwise comparisons when found to be significant at $P < 0.05$. The percentage efficacy (PE) of each treatment was calculated by means of the standard equation: (1-(T/C)) X 100 where C and T are the arithmetic mean total nematode burdens or FECs (on day 35 PI) of the untreated control and treated groups, respectively (Coles et al., 1992). Anthelmintic resistance was deemed to be present when the PE in reducing FEC was < 95%, with a lower 95% confidence limit of < 90%.

All experimental procedures described here were approved by the Moredun Research Institute Experiments and Ethics Committee and were conducted under the legislation of a UK Home Office License (reference P95890EC1) in accordance with the Animals (Scientific Procedures) Act of 1986.

3. Results

3.1. Faecal egg counts

Faecal egg counts rose in all of the animals from day 14 post infection until day 28 PI, peaking at an arithmetic mean of 364 eggs per gram (range 24–1044). The arithmetic mean FECs of the treatment group fell following monepantel administration on day 28 PI, but rebounded thereafter (Fig. 1). FECs plateaued in the untreated control group. Efficacy based on undifferentiated FEC was 85% (95% CI 61% 94%) on day seven post monepantel administration. A significant difference ($p = 0.04$) in FEC compared to non-treated control animals at day seven post treatment was observed.

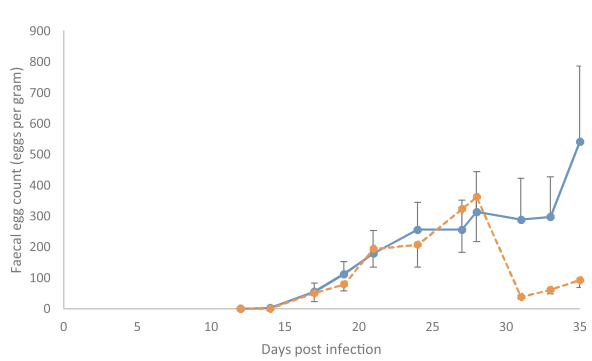

Fig. 1. Faecal egg counts (± standard error of the mean) of lambs artificially infected with 15,000 infective larvae of field derived isolate. Lambs were either administered monepantel (dashed line) on day 28 post infection or left untreated (solid line) as controls.

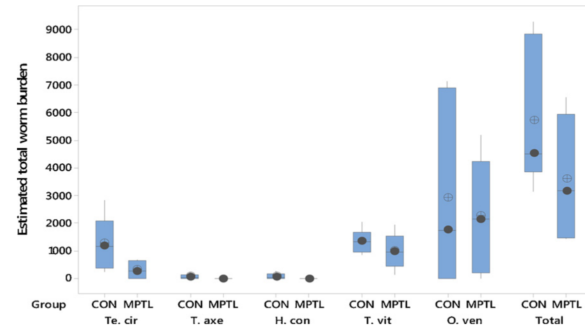

Fig. 2. Box-plot of estimated worm burden counts of untreated control (CON) lambs relative to monepantel treated (MPTL) lambs 7 days post treatment. Te. cir = *Teladorsagia circumcincta*; T. axe = *Trichostrongylus axei*; H. con = *Haemonchus contortus*; O. ven = *Oesophagostomum venulosum*; Total = total worm burden. Box = 25th and 75th percentiles with whiskers = maximum and minimum values, ⊕ = mean and ● = median.

3.2. Nematode burden analysis

The arithmetic mean percentage establishment of nematodes in the control lambs was 38%, 5717 nematodes recovered from 15,000 used to establish infection. In terms of species composition identified at post mortem the untreated control comprised 22% *Teladorsagia circumcincta*, 1% *Trichostrongylus axei*, 2% *Haemonchus contortus*, 24% *Trichostrongylus vitrinus* and 51% *Oesophagostomum venulosum* (Fig. 2). Monepantel efficacies, when compared to the untreated control group, were 100%, 100%, 78%, 27% and 22% for *T. axei, H. contortus, T. circumcincta, T. vitrinus,* and *O. venulosum* respectively.

4. Discussion

This study findings demonstrated that treatment with the manufacturer's recommended dose rate of 2.5 mg/kg bodyweight of monepantel was highly effective against *H. contortus* and *T. axei* (100%), albeit that only small numbers of adults of these species were detected in the untreated control lambs, but that three parasite species, *T. circumcincta, T. vitrinus* and *O. venulosum* were expressing resistance. The outcome confirms previous unproven suspicions that more than one resistant genus was present post monepantel administration on this farm (Hamer et al., 2018).

Historically the first reports of resistance to broad-spectrum anthelmintic drugs have often been monogeneric in nature (Conder and Campbell, 1995), generally involving *H. contortus* (Smeal et al., 1968) or *T. circumcincta* (Hall et al., 1979) although cases of multigeneric resistance are not unprecedented. Initial reports of monepantel resistance globally have implicated a number of parasite genera, namely *T. circumcincta* (Scott et al., 2013), *T. colubriformis* (Scott et al., 2013), *H. contortus* (Mederos et al., 2014; Van den Brom et al., 2015) and *Oesophagostomum* species (Cintra et al., 2016).

The finding of multigeneric resistance may reflect the dynamic and diverse nature of nematode populations within livestock, complex animal movements within and between farms, and the widespread use of anthelmintic drugs for helminth control. So how might resistance in multiple species arise in a very short timeframe? Two routes of multigeneric resistance development have been proposed. Firstly, changes in nematode epidemiology and disease patterns influenced by farming practices, anthelmintic resistance and/or climate change (Van Dijk et al., 2009) may allow anthelmintic drug exposure of some species at times and frequencies previously unseen. Secondly, due to the mobile nature of animals, and as a result of imperfect quarantine treatment strategies adopted by many farmers (Coles, 2003), it is possible that resistance by the different parasite species has been selected independently and transferred through animal movements. In the absence of contradictory evidence, our findings highlight the need for strict quarantine treatments for all new and returning stock and to ensure that the anthelmintic drug combinations used are fully effective through post quarantine drench efficacy tests.

The parasite make-up of the resistant population is interesting given the previous reports of monepantel resistance globally, where all of the species, barring *T. vitrinus*, have been implicated. Within the UK, surveys of resistance to other classes of anthelmintic have commonly identified *T. circumcincta* (Bartley et al., 2006); occasionally identified *Trichostrongylus* (Bartley et al., 2006), but rarely *Oesophagostomum*. *Oesophagostomum venulosum* was identified as one of the dose limiting species for monepantel with variable results (efficacy ranging between 86.8 and 96.5%) between the different trials conducted by Novartis AH (Hosking, 2010). Although equivocal results have previously been reported against *Oesophagostomum* it is unlikely that the lack of efficacy observed in this study is solely the result of an innate insensitivity to the compound, but these findings merit further investigation.

The findings of this current study need to be kept in perspective, although multigeneric resistance was identified and reduces future treatment options on the index farm, this is currently an isolated case. Further study would be required to assess the scale of the problem in UK flocks. Nevertheless, the results highlight the importance of maintaining the awareness of producers to the possibility of anthelmintic resistant nematodes in sheep and the need to follow appropriate guidelines when it comes to quarantine treatment of new and returning stock and the merit of testing the efficacy of treatments on a regular basis to ensure that they are effective.

Declaration of interests

The authors declare that there is no conflict of interest.

Acknowledgements

We gratefully acknowledge funding from The Scottish Government's Rural and Environment Science and Analytical Services Division (RESAS). We are grateful to the Bioservices Division, Moredun Research Institute, for expert care and assistance with animals and the original farmer for supplying access to animals for collection of faecal material.

References

Bartley, D.J., Donnan, A.A., Jackson, E., Sargison, N., Mitchell, G.B.B., Jackson, F., 2006. A small scale survey of ivermectin resistance in sheep nematodes using the faecal egg count reduction test on samples collected from Scottish sheep. Vet. Parasitol. 137, 112–118.

Christie, M., Jackson, F., 1982. Specific identification of strongyle eggs in small samples of sheep faeces. Res. Vet. Sci. 32, 113–117.

Cintra, M.C., Teixeira, V.N., Nascimento, L.V., Sotomaior, C.S., 2016. Lack of efficacy of monepantel against *Trichostrongylus colubriformis* in sheep in Brazil. Vet. Parasitol. 216, 4–6.

Coles, G., 2003. Strategies to minimise anthelmintic resistance in large animal practice. In Pract. 25, 494–499.

Coles, G.C., Bauer, C., Borgsteede, F.H.M., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44.

Conder, G.A., Campbell, W.C., 1995. Chemotherapy of nematode infections of veterinary importance, with special reference to drug-resistance. Adv. Parasitol. 35 (35), 1–84.

Hall, C.A., Campbell, N.J., Carroll, S.N., 1979. Resistance to thiabendazole in a field population of *Ostertagia circumcincta* from sheep. Aust. Vet. J. 55, 229–231.

Hamer, K., Bartley, D., Jennings, A., Morrison, A., Sargison, N., 2018. Lack of efficacy of monepantel against trichostrongyle nematodes in a UK sheep flock. Vet. Parasitol. 257, 48–53.

Hosking, B.C., 2010. The Control of Gastro-Intestinal Nematodes in Sheep with the Amino-Acetonitrile Derivative, Monepantel with Particular Focus on Australia and New Zealand. Ghent University, Ghent, Belgium.

Hosking, B.C., Kaminsky, R., Sager, H., Rolfe, P.F., Seewald, W., 2010. A pooled analysis of the efficacy of monepantel, an amino-acetonitrile derivative against gastro-intestinal nematodes of sheep. Parasitol. Res. 106, 529–532.

Jabbar, A., Iqbal, Z., Kerboeuf, D., Muhammad, G., Khan, M.N., Afaq, M., 2006. Anthelmintic resistance: the state of play revisited. Life Sci. 79, 2413–2431.

Leathwick, D.M., Hosking, B.C., 2009. Managing anthelmintic resistance: modelling strategic use of a new anthelmintic class to slow the development of resistance to existing classes. N. Z. Vet. J. 57, 203–207.

MAFF, 1986. Ministry of Agriculture, Fisheries and Food, Manual of Veterinary Parasitological Laboratory Techniques, Reference Book 418, third edition. Her Majesty's Stationery Office.

Mederos, A.E., Ramos, Z., Banchero, G.E., 2014. First report of monepantel *Haemonchus contortus* resistance on sheep farms in Uruguay. Parasites Vectors 7, 598. https://doi.org/10.1186/s13071-014-0598-z.

Playford, M.C., Smith, A.N., Love, S., Besier, R.B., Kluver, P., Bailey, J.N., 2014. Prevalence and severity of anthelmintic resistance in ovine gastrointestinal nematodes in Australia (2009–2012). Aust. Vet. J. 92, 464–471.

Sager, H., Rolfe, P., Strehlau, G., Allan, B., Kaminsky, R., Hosking, B., 2010. Quarantine treatment of sheep with monepantel—rapidity of fecal egg count reduction. Vet. Parasitol. 170, 336–339.

Scott, I., Pomroy, W.E., Kenyon, P.R., Smith, G., Adlington, B., Moss, A., 2013. Lack of efficacy of monepantel against *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Vet. Parasitol. 198, 166–171.

Smeal, M.G., Gough, P.A., Jackson, A.R., Hotson, I.K., 1968. The occurrence of strains of *Haemonchus contortus* resistant to thiabendazole. Aust. Vet. J. 44, 108–109.

Van den Brom, R., Moll, L., Kappert, C., Vellema, P., 2015. *Haemonchus contortus* resistance to monepantel in sheep. Vet. Parasitol. 209, 278–280.

Van Dijk, J., Sargison, N.D., Kenyon, F., Skuce, P.J., 2009. Climate change and infectious disease: helminthological challenges to farmed ruminants in temperate regions. Animal 4 (3), 377–392.